BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

100

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B01J 19/00 — A1

(11) International Publication Number: WO 97/37755

(43) International Publication Date: 16 October 1997 (16.10.97)

(21) International Application Number: PCT/US97/05841

(22) International Filing Date: 9 April 1997 (09.04.97)

(30) Priority Data:

08/630,018	9 April 1996 (09.04.96)	US
08/730,636	11 October 1996 (11.10.96)	US
08/744,386	7 November 1996 (07.11.96)	US

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(71) Applicant: SARNOFF CORPORATION [US/US]; 201 Washington Road, CN 5300, Princeton, NJ 08543 (US).

(72) Inventors: DEMERS, Robert, Richard; 26 Evans Drive, Cranbury, NJ 08512 (US). CHERUKURI, Satyam, Choudary; 90 Cranbury Neck Road, Cranbury, NJ 08512 (US). LEVINE, Aaron, William; 6 Springwood Drive, Lawrenceville, NJ 08648 (US). O'MARA, Kerry, D.; 308 West Second Street, Florence, NJ 08518 (US).

(74) Agents: BURKE, William, J. et al.; Sarnoff Corporation, 201 Washington Road, CN 5300, Princeton, NJ 08543-5300 (US).

(54) Title: PLATE FOR REACTION SYSTEM

(57) Abstract

The invention provides a plate having a plurality of uniformly sized reaction cells formed in its upper surface, wherein the density of the reaction cells is at least about 10 cells per cm^2. Preferably, the area of each of the openings of the reaction cells is no more than about 55 % of the area defined by the multiplication product of (1) the pitch between reaction cells in separate rows and (2) the pitch between reaction cells in separate columns.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

PLATE FOR REACTION SYSTEM

The present application is a continuation-in-part of U.S. Application 08/630,018, filed April 9, 1996, titled "Plate for Reaction System," U.S. Application 08/730,636, filed October 11, 1996, titled "Liquid Distribution System and U.S. Application 08/744,386, filed November 7, 1996, titled "Liquid Distribution System."

The present invention relates to a plate having formed thereon numerous small-scaled reaction cells (or wells) that are formatted to allow the cells to be individually addressed despite their small size, to allow each cell to accommodate a substrate for reactions, to allow each cell to be separately illuminated for optical detection, and to allow sufficient material between reaction cells to support the formation of an appropriate seal between the plate and an ancillary device, for instance a liquid distribution device that delivers reagents to the reaction cells.

Recent advances in microfluidics, i.e., the small-scale transfer of liquid among compartments, have made it possible to conduct reactions such as syntheses or assays in very small-scale devices. See, for instance, Zanzucchi et al., "Liquid Distribution System," U.S. Patent Application No. 08/556,036, filed November 9, 1995. Such devices allow the programming of the concurrent operation of thousands of separate reactions. Pursuant to the Zanzucchi et al. patent application, such an apparatus can be used to distribute various liquids to thousands of reaction cells, which reaction cells can be fabricated on a plate.

In recent years, drug discovery programs have focused on the use of microtiter plates having 96 or 384 cells to assay prospective pharmaceuticals in an *in vitro* model for a pharmaceutical activity or for conducting small-scale syntheses in parallel. The devices provided for by the advances in microfluidics, however, are to be used to conduct reactions at, for example, 1,000, 10,0000, 100,000 or more reaction sites or cells. By the present invention, these sites or cells are located on a plate. In attempting to operate at the scale implied by these numbers of reaction cells, one must take into account, among other things, (1) the need to form seals that allow fluid to be transferred to each cell without cross-contamination from fluid intended for another cell, (2) the need to provide a cell aperture widths sufficient to facilitate fluid transfer, (3) the need for the cells to have

sufficient cross-sectional area and volume to allow for optical detection, (4) the need to have a relatively compact plate that is easily stored and operates with a liquid distribution device that moves liquids through relatively compact distances and (5) the need for sufficient alignment and cell separation to allow the cells to be individually identified. By the present invention, these needs are met with a "nanotiter" or "small-scaled" plate having reaction cells that are densely packed.

SUMMARY OF THE INVENTION

The invention provides a plate having a plurality uniformly sized reaction cells formed in its upper surface, wherein the density of the reaction cells is at least about 10 cells per cm^2. Preferably, the reaction cells are arrayed in rows and columns. Also, preferably, the plate is rectangular, preferably with the rows and columns of cells parallel to the edges of the plate. Preferably, the area of each of the openings (i.e., apertures) of the reaction cells is no more than about 55% of the area defined by the multiplication product of (1) the pitch between reaction cells in separate rows and (2) the pitch between reaction cells in separate columns. The product between this row pitch and column pitch can be termed the "footprint" of a cell, meaning the amount of area supporting each cell of a plate. More preferably, this aperture area is no more than about 50%, yet more preferably 45%, of the cell footprint. Preferably, the density of cells is no more than about 350 per cm^2, more preferably no more than about 150 per cm^2, yet more preferably no more than about 120 per cm^2. Preferably, the density of cells is at least about 10 cells per cm^2, more preferably at least about 20 cells per cm^2, more preferably at least about 40 cells per cm^2, still more preferably at least about 100 cells per cm^2.

The footprint, which is symmetrically overlaid on the cell, encompasses plate surface area on all sides of a cell. The minimum distance from the edge of the cell to the boundary of the footprint is here termed the "seal strip width," since this is the area on which a gasket material can be applied. In one preferred embodiment, the density of cells is from about 10 to about 45 cells per cm^2, more preferably about 10 to about 20 cells per cm^2, and the seal strip width is from about 300 μm to about 1,000 μm, more preferably from about 600 μm to about 1,000 μm.

Preferably, the diameter or width of the aperture of a cell is about 400 μm to about 1100 μm, more preferably about 900 μm to about 1100 μm. The depth of the cells

is preferably from about 100 μm to about 400 μm, more preferably about 250 μm to about 350 μm.

Preferably, on the plate, the pitch between reaction cells in a row or column is at least about 0.5 mm, more preferably at least about 0.9 mm. Preferably, each reaction cell is separated from each adjacent reaction cell by at least about 0.15 mm, more preferably by at least about 0.3 mm. Preferably, each reaction cell has a substantially square shape. Preferably, the plate has at least about 1,000 reaction cells, more preferably at least about 4,000 reaction cells, yet more preferably at least about 10,000 reaction cells. Preferably, the plate has a patterned gasket on its upper surface.

Preferably, the plate is designed to facilitate alignment by having a first marker on a first edge of the plate, wherein the marker is for orienting the reaction cells. Preferably, the plate has a second marker on a second edge of the plate perpendicular to the first edge, wherein the second marker is for orienting the reaction cells. More preferably, the plate has a third marker on the second edge, wherein the third marker is for orienting the of reaction cells. Preferably, the first, second and third markers are notches designed to interact with locating pins used to mechanically orient the reaction cells. Alternatively or supplementally, the plate has two optical reference structures, more preferably three, for orienting a device, such as an optical detector, relative to the reaction cells. The optical reference structures are preferably separated by at least about 4 cm. Preferably, the optical reference structures are etched into the plate.

The invention also provides a reaction system for conducting a plurality of reactions in parallel, the reaction system comprising a liquid distribution system for addressably directing a plurality of liquids to a plurality of cells, and a plate as described above.

The invention additionally provides a method of conducting a plurality of reactions in parallel comprising operating a liquid distribution system for addressably directing a plurality of liquids to a plurality of cells, wherein the cells are located on one of the above-described plates that is reversibly sealed to the liquid distribution system.

The invention further provides a method of forming a gasket on the top surface of a substrate, the method comprising: patterning a layer of photoresist on the top surface so that there are cleared surface areas and photoresist-coated areas; applying an elastomeric gasket material to the cleared areas; and removing the photoresist from the

photoresist-coated areas. Preferably, the applying step comprises placing the elastomeric gasket material on the top surface; and compression-molding the gasket material into the cleared areas. Preferably, after the compression-molding and before the photoresist removal, the gasket material is cured. The gasket material is preferably silicone. The substrate preferably has a smooth surface, more preferably a flat surface. The method is preferably applied to a plate on which structures, particularly microstructures, have been formed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts four formats for the plate of the invention.

Figure 2 shows schematically some of the parameters considered in designing the format of the plate of the invention.

Figures 3A-3C show three cell designs.

Figure 4 illustrates a plate of the invention connected to a liquid distribution system.

Figure 5 shows a plate of the invention.

Figure 6A and 6B illustrate a portion of a gasket print pattern.

DEFINITIONS

The following terms shall have the meaning set forth below:

- **addressable**

Cells are addressable if each cell of a plate can be individually located and its contents manipulated.

- **alignment**

"Alignment" refers to the coordinated positioning of the cells of a small-scaled plate of the invention and another device with which the small-scaled plate will operate. Thus, the small-scaled plate is aligned with a liquid distribution system if the liquid distribution system is positioned to deliver liquid to each of the cells of the small-scaled plate. Similarly, the small-scaled plate is aligned with an optical detection device if the device can focus light on each cell and separately identify the transmission or fluorescence of each cell. During fabrication of the small-scaled plate, the concept relates to the relative positioning between a device, such as a fabrication device, and the design location of the cells of the small-scaled plate as set forth in the design plans.

• **micromachining**

"Micromachining" is any process designed to form microstructures on a substrate.

structure

A "structure" is formed on the upper surface of a plate is a shape defined by variations in the elevation of the upper surface. Preferably, structures are "microstructures" having dimensions of about 2 mm or less.

DETAILED DESCRIPTION

The small-scaled plate 100 of the invention is formed of a substrate that is an organic or inorganic material that is suitable for forming the fine structures described herein. The small-scaled plate 100 should be formed of a material that is resistant to the types of materials it is anticipated to encounter in use. Thus, for instance, in diagnostic settings the small-scaled plate 100 typically encounters aqueous materials and can, accordingly, be manufactured of a broad range of materials. Where the small-scaled plate 100 is designed for use in synthetic reactions, often the 100 should be constructed of a material that is resistant to acids, bases and solvents. In one preferred embodiment, the small-scaled plate 100 is constructed of glass, particularly borosilicate glass.

A basic parameter for the small-scaled plate 100 is the spacing between the centers of adjacent cells 101, which spacing is termed the "pitch." Four cell formats for plates are illustrated in Figure 1; these formats are the 1K, 4K, 10K and 100K formats. The 1K format has a pitch of 2260 μm; the 4K format has a pitch of 1488 μm; the 10K format has a pitch of 965 μm; and the 100K format has a pitch of 558 μm. Illustrative parameters for these formats are set forth below:

FORMAT	1K	4K	10K	100K
NUMBER OF CELLS	32 X 32 = 1024	64 X 64 = 4096	100 X 100 = 10,000	316 X 316 ≈100,000
SUBSTRATE SIZE	3 inch square	4 inch square	4 inch square	7.25 inch square
CELL SIZE	890 µm square	890 µm square	635 µm square	635 µm square
CELL PITCH	2260 µm	1488 µm	965 µm	558 µm
MIN. CELL VOLUME	120 nL	120 nL	50 nL	10 nL
MIN. CELL DEPTH	200 µm	200 µm	200 µm	150 µm

Further illustrative parameters for such formats are set forth below:

FORMAT	144	864	3,456	10,368
NUMBER OF CELLS	12 X 12 = 144	36 X 24 = 864	72 X 48 = 3,456	144 X 72 = 10,368
SUBSTRATE SIZE (inches)	1.417 inch square	3.36 X 5.03	6.72 X 10.06	7.25 inch square
CELL SIZE	1,000 µm square	1,000 µm square	1,000 µm square	1,000 µm square
CELL PITCH	3,000 µm	3,000 µm	3,000 µm	3,000 µm
MIN. CELL VOLUME	300 nL	300 nL	300 nL	300 nL
MIN. CELL DEPTH	300 µm	300 µm	300 µm	300 µm

In the illustration, cell volume and depth are selected to help accommodate the insertion of beads on which synthetic or other chemistries are conducted.

Focusing on the 1K format, the pitch is the 2260 µm distance illustrated in Figure 1. The area defined by the pitch further defines the amount of surface area that a given cell 101 resides within. Thus, the product of the pitch between cells 101 in a row and the

pitch between cells 101 in a column determines the size of the surface area on which an individual cell 101 sits. The percentage of this surface area taken up by the area of each of the cell apertures is the area of the cell openings divided by the above-described product, times 100%.

It is useful in understanding how the small-scaled plate is used, to refer to Zanzucchi et al., "Liquid Distribution System," U.S. Patent Application No. 08/556,036, filed November 9, 1995, which application is incorporated herein in its entirety by reference. This patent application describes a liquid distribution system ("LDS") that can deliver fluid from a number of reservoirs to all of a set of reaction cells that are connected to the LDS and from additional reservoirs to a substantial subset of these reaction cells. The liquid distribution device is designed for use in applications requiring a high density of reaction cells. In a preferred embodiment, the device uses electrode-based pumps that have no moving parts to transport fluid from the reservoirs to the reaction cells. The reaction cells are preferably found on a plate 100 that is separable from the portion of the liquid distribution system containing reservoirs and pumps. The separable plate 100 docks with the liquid distribution system, typically with a gasket material (that has openings at appropriate locations) interposed between the two, so that the cells are aligned underneath the appropriate outlet for delivering liquid from the liquid distribution system.

Three parameters that are basic to the format of the plate 100 are the spacing between cells 101 (i.e., pitch), the area of each of the openings of the cells 101 which will be referred to as the cell aperture, and the row-column arrangement which will be referred to as the matrix layout. The depth of a cell 101 can be made to vary according to the application for which the plate 100 is used. Structures required for support functions can be formed on the area between cell apertures.

The determination of pitch is based on factors including the following:

- size of the substrate;
- surface area required by the cell aperture;
- allowing adequate surface area for the mechanical and electrical architecture for features and devices that direct and control fluids to be introduced into the cells 101;

- allowing adequate surface area for sealing to ensure the isolation of fluids;
- allowing for practical mechanical resolution in processes for loading and unloading materials such as fluids, beads and pellets;
- compatibility with a means of illuminating each cell 101 in an individual and addressable manner for the reaction detection process;
- compatibility with a means of sensing, for each cell 101 individually, that a desired reaction has taken place;
- compatibility with fabrication techniques such as photolithography, micromachining, electroforming and pressure molding; and
- structural integrity of the substrate.

The determination of cell aperture is based on factors including the following:

- selecting an appropriate size to provide an adequate reagent fluid volume needed for the chemical reaction that is designed to take place in the cell 101;
- selecting an adequate size to provide reliable flow of reagent fluids through the cell 101, where this determination should take into account the possibility that the cell 101 contains a solid support media or bead 102;
- limitations on available surface area due to the selection of the best compromise between smaller cell pitch (thus greater cell density) and larger cell aperture (thus, greater cell volume and accessibility);
- allowing for entry by instruments for loading and unloading materials such as fluids, beads and pellets;
- allowing the cell to be accessed by reagents used to add a functionality to the surface of the cell, such as siliconizing agents used to minimize surface adsorption or control the wetting properties of the surface;
- compatibility with a means for illuminating each cell 101 individually;

- compatibility with a means for detecting reaction in each cell 101 individually;
- compatibility with fabrication techniques such as photolithography, micromachining, electroforming and pressure molding; and
- structural integrity of the substrate.

The determination of the matrix layout is based on factors including the following:

- the needs of the experimental, diagnostic, screening or synthesis procedure to be conducted in the small-scaled plate 100;
- the need to efficiently use the surface area of the substrate;
- efficiency and density of the reagent fluid circuit of the liquid distribution system that interacts with the small-scaled plate;
- convenient addressability of each cell 101;
- compatibility with a means for illuminating each cell individually; and
- compatibility with a means for detecting reaction for each cell individually.

An algorithm used for addressing the best compromise of these variables is illustrated graphically in Figure 2, with the connecting lines indicating inter-related concepts relating to cell pitch, cell aperture and matrix layout that should be considered in arriving at a format..

Designs of particular interest can be met by the matrix formats of 1,000 cells 101 represented by a matrix of 32 x 32=1,024 cells 101; 4,000 cells 101 represented by a matrix of 64 x 64=4,096 cells 101; and 10,000 cells 101 represented by a matrix of 100 x 100=10,000 cells 101. Such Designs are illustrated in Figure 1. Intermediate formats covering a different number of cells 101, and asymmetric matrix layouts can also be fabricated. Some design considerations that went into the formats of Figure 1 are outlined below.

Format 1 K

Format 1 K is a 1024 cell array symmetrically formed into 32 rows and 32 columns and having a reaction cell volume of at least about 120 nanoliter per cell 101. The substrate size has been selected by balancing the pressures toward minimum size

imposed by handling and fluidics factors and the pressures for maximum size imposed by ease of fabrication considerations. Using approaches that most reflect the performance implied by this format, a substrate size of 3 inch x 3 inch has been selected as the best compromise. For this size and array configuration, in a typical case, a cell pitch of 2260 μm can be accommodated. A cell configuration that satisfies volumetric and surface area requirements for fluid delivery, synthesis, assay and detection is 890 μm x 890 μm. Using typical micromachining techniques suitable for production (for example see the description below of such a technique), the cells 101 have a fluid capacity of a minimum of 120 nanoliters.

Format 4K

Format 4K is a 4096 cell array symmetrically formed into 64 rows and 64 columns and having a reaction cell volume capacity of at least about 120 nanoliter per cell 101. As above, a compromise between operation, handling and fabrication has led to the selection of a substrate size of 4 inch x 4 inch. For this size and array configuration, in a typical case, a cell pitch of 1488 μm can be accommodated. The cell configuration of 890 μm square of the 1K format, which configuration satisfies volumetric and surface area requirements for fluid delivery, synthesis, assay, and detection, can be maintained. Using typical micromachining techniques suitable for production, the cells 101 have a fluid capacity of a minimum of 120 nanoliters.

Format 10K

Format 10K is a 10,000 cell array symmetrically formed into 100 rows and 100 columns. A maximum of 4 inch x 4 inch substrate size was selected for handling and fabrication reasons. Micromachined features are reduced in size from the 4K cell format. For use with this 10K plate, the associated liquid distribution system, for instance a liquid distribution system according to Zanzucchi et al.,"Liquid Distribution System," U.S. Patent Application No. 08/556,036, filed November 9, 1995, is also fabricated with a correspondingly dense layout of fluid delivery capillaries. With such a dense layout of fluid delivery capillaries, a cell pitch of 965 μm in the small-scaled plate can be accommodated. The cell configuration is adjusted for the more demanding requirements created by the higher density of cells. A useful resolution of the volumetric and surface area requirements for fluid delivery, synthesis, assay and detection, is 635 μm x 635 μm

cell aperture. Using micromachining techniques suitable for production, the cells 101 have a fluid capacity of a minimum of 50 nanoliters.

The reaction cell aperture is preferably substantially square or rectangular in profile to best accommodate an array format. The aperture can have rounded corners to accommodate the micromachining or molding/replication techniques used. Thus, "substantially" in this context means no more than the amount of rounding or irregularity in shape that can be expected when such structures are formed in glass by chemical etching, as predominately practiced commercially in 1995. Preferably, the circular features formed at the edges of the "rectangular" or "square" cells have radii no greater than the depth of the cell and the edges of the aperture of the cell are longer than the cell depth.

In Figure 3, above line A are shown three top views for three different cell 101 designs (first cell 101A, second cell 101B and third cell 101C). Below line A are shown the side profiles of first cell 101A, second cell 101B and third cell 101C. The profile of first cell 101A illustrates the relatively sharp edge lines obtained by chemically etching a silicon substrate. The profile of second cell 101B illustrates the relatively sharp edge lines obtained by laser etching a glass substrate. When chemically etching a glass substrate, the lines obtained are typically less sharp, as illustrated in Figure 3C. The cell 101 cross-sectional profile can be of various shapes depending on the micromachining or replication technique but should preferably meet a minimum fluid volume capacity and must provide enough depth to accommodate experiments that require a bead 102 for use in syntheses or assays that require a solid support. Although a number of beads per cell may be used, and although beads of different sizes may be used depending on the experiment, the preferred design consideration is based on providing adequate space for synthesis or other reaction on a single bead of a defined maximum specified swollen diameter. In one use of the nanotiter plates, cell depths sufficient to accommodate swollen beads of about 200 μm diameter, or even about 400 μm, are used in formats 1K, 4K; and depths sufficient to accommodate swollen beads of 100 μm diameter are used in format 100K.

The cell profile is achieved with micromachining, replicating, molding, or like fabrication methods, cells in a single substrate, or is achieved by combining multiple layers of substrates. The combining of layers can be achieved by known methods or, with

appropriate substrates, with the field-assist sealing method described in Zhonghui H. Fan et al., U.S. Provisional Application No. P-89,876, titled "Field Assisted Glass-Glass Sealing," filed November 7, 1995, which is incorporated herein in its entirety by reference. When the small-scaled plate is used for detection, optical requirements are important variables in the selection of cell construction, cross-sectional profile, and material. The small-scaled plate allows for the space between cells to be used to provide for fluid conduits and drains, electrical vias, sealing features, and the like. The small-scaled plate can be constructed of any materials, material combinations, substrate thicknesses, and fabrication techniques, that suit the application.

Figure 4 illustrates one way the surface area between cell apertures can be used. The small-scaled plate 320 is formed of a single plate and has formed thereon cells 350. The small-scaled plate 320 is designed for use with a liquid distribution system ("LDS") formed of first LDS plate 300 and second LDS 310. Liquid is delivered to each cell 350 through a first conduit 390. Excess fluid flows out through second conduit 355, which connects to cell 350 through third conduit 351.

Provision is preferably made on the small-scaled plate 100 to facilitate alignment (a) with the apparatuses that fabricate the small-scaled plate, and during assembly (b) with liquid distribution systems and other processing or detection equipment. For many cases mechanical alignment using three-pin registry is acceptable, and the edge alignment locations specified in Figure 5 can be used. Although other alternatives can be used, the preferred method is to grind first edge notch 105A, second edge notch 105B and third edge notch 105C, for instance at the locations shown in Figure 5. The use of such notches obviates the need to accurately machine all the edges of the small-scaled plate 100 and provides for a method of mechanically identifying the top and bottom of the plate 100. The location of the center of the cell patterns is defined in Figure 5 by the intersection of lines B and C. The use of comparable notches in the manufacture of a liquid distribution system with which the small-scaled plate operates allows equipment and tool manufacturers to coordinate their designs.

In the illustrated small-scaled plate of Figure 5, examples of the distances represented by R1, R2, R3, Cm and Co are:

FORMAT	R1	R2	R3	Cm	Co
1K	0.25 in	2.70 in	2.70 in	1.45 in	1.25 in
4K	0.25 in	3.70 in	3.70 in	1.95 in	1.75 in
100K	0.25 in	6.95 in	6.95 in	3.57 in	3.37 in

In some cases, optical alignment is preferable. The preferred location for the optical fiducials, such as first fiducial 106A, second fiducial 106B and third fiducial 106C, are illustrated in Figure 5.

For all of the above-described embodiments, the preferred support material will be one that has shown itself susceptible to microfabrication methods, such as a microfabrication method that can form channels having cross-sectional dimensions between about 50 microns and about 250 microns. Such support materials include glass, fused silica, quartz, silicon wafer or suitable plastics. Glass, quartz, silicon and plastic support materials are preferably surface treated with a suitable treatment reagent such as a siliconizing agent, which minimizes the reactive sites on the material, including reactive sites that bind to biological molecules such as proteins or nucleic acids. In embodiments that require relatively densely packed electrical devices, a non-conducting support material, such as a suitable glass, is preferred. Preferred glasses include borosilicate glasses, low-alkali lime-silica glasses, vitreous silica (quartz) or other glasses of like durability when subjected to a variety of chemicals. Borosilicate glasses, such as Corning 0211, 1733, 1737 or 7740 glasses, available from Corning Glass Co., Corning, NY, are among the preferred glasses.

The reaction cells and horizontal channels and other structures of the small-scaled plates can be made by the following procedure. A plate is coated sequentially on both sides with, first, a thin chromium layer of about 500Å thickness and, second, a gold film about 2000 angstroms thick in known manner, as by evaporation or sputtering, to protect the plate from subsequent etchants. A two micron layer of a photoresist, such as Dynakem EPA of Hoechst-Celanese Corp., Bridgewater, NJ, is spun on and the photoresist is exposed, either using a mask or using square or rectangular images, suitably

using the MRS 4500 panel stepper available from MRS Technology, Inc., Acton, MA. After development to form openings in the resist layer, and baking the resist to remove the solvent, the gold layer in the openings is etched away using a standard etch of 4 grams of potassium iodide and 1 gram of iodine (I_2) in 25 ml of water. The underlying chromium layer is then separately etched using an acid chromium etch, such as KTI Chrome Etch of KTI Chemicals, Inc., Sunnyvale, CA. The plate is then etched in a bath, such as an ultrasonic bath, of HF-$HN0_3$-H_20 in a ratio by volume of 14:20:66. The use of this etchant, for example in an ultrasonic bath, produces vertical sidewalls for the various structures. Etching is continued until the desired etch depth is obtained. Vertical channels are typically formed by laser ablation.

The gasket used to reversibly seal the plate to an instrument that functions with the plate can be attached to the plate, leaving openings for the cells and other structures, as needed. One method of attaching the gasket is screen-printing. The printed gasket can be made of silicone or another chemically-resistant, resilient material.

Alternatively, a multi-step compression-molding process that utilizes photolithography can be applied. First, the top surface of the plate, on which generally cells and other structures have been formed, is coated with a photoresist. Preferably, the photoresist layer is about 1 mil in thickness. The photoresist layer is treated by standard photolithography techniques to remove photoresist from those areas (the "gasket areas") away from the apertures of the cells where gasket material is desired. A layer of a flowable gasket material that can be cured to a resilient, elastomeric solid is applied. A platen having a polished surface, for instance a polished glass surface, is placed above the gasket material and pressure is applied to push the gasket material into the gasket areas and substantially clear the gasket material from the photoresist-coated areas. The gasket material is now cured. The photoresist is then dissolved, leaving the plate with a patterned gasket. The gasket material is substantially cleared if it is sufficiently cleared to allow the underlying photoresist to be dissolved.

In this process, the gasket material is any elastomeric material that is suitable for use in the above-described compression molding technique, that is, when cured, compatible with the chemistries that are to be practiced in the plate on which the gasket is formed, and that is, when cured, resistant to the solvents used to remove the photoresist.

The gasket material is preferably silicone, such as RTV type silicone rubber (e.g., Silastic J, RTV Silicone Rubber available from Dow Corning, Midland, Michigan). The photoresist can be a film-type photoresist such that typically the structures on the plate will not be filled during the compression-molding process or a liquid-type photoresist such that the structures will temporarily be filled during the compression-molding process and etched away at the completion of the process. In some instances, it is desirable to treat the plate, prior to the application of the photo-resist, with a primer for promoting the adhesion of the gasket material, such as 1200 RTV Prime Coat from Dow Corning, Midland, Michigan. The plate can also be roughened to promote the adhesion of the gasket material to the plate. For example, 5 micron roughness can be produced by lapping. The platen is preferably treated with a release-promoter, or a release promoter is incorporated into the gasket material, as it is in Silastic J silicone rubber. The compression-molding process can leave thin residues of gasket material at unwanted locations. These residues are laser cut away from the plate or, in some cases, are removed using a timed exposure to a solvent that dissolves the thin film of exposed gasket material residue without having substantial effect on the thicker layer of gasket material found at desired locations.

Another method of attaching the gasket is screen-printing. The printed gasket can be made of silicone or another chemically-resistant, resilient material. Preferably, the gasket is made of a mixture of (a) a silicone rubber-forming material such as that available under the Sylgard 184™ brand from Dow Corning, Midland, Michigan or MDX4-4210™ also from Dow Corning and (b) an inert filler, such as the amorphous fumed silicon sold as M-5 grade Cab-o-sil™ (Cabot Corp., Boston, MA). Sylgard 184 and MDX4-4210 are sold in two components. One component is an emulsion containing particles of silcone rubber and a polymerization catalyst and the second component is a preparation of a bi-valent monomer, which monomer serves to crosslink and thereby cure the silicone rubber. Component one of MDX4-4210, i.e. the "elastomer component," is made up of dimethylsiloxane polymer, reinforcing silica, and a platinum catalyst. Component two of MDX4-4210, the "curing agent," also contains dimethylsiloxane polymer, in addition to a polymerization inhibitor, and a siloxane crosslinker. The components are generally mixed according to the manufacturer's recommendations. For example, for MDX4-4210, ten

parts by weight of emulsion, i.e. elastomer, are mixed with one part of monomer solution, i.e. curing agent.

As examples of the use of inert fillers, about 7.5% by weight of M-5 grade Cab-o-sil can be added to the Sylgard 184, or about 2-3% by weight of M-5 grade Cab-o-sil can be added to the MDX4-4210. Filler can serve to thicken the pre-polymerized composition to improve its screen printing properties. Gasket materials can generally be cured at room temperature, or curing, can be accelerated with, for example, heat. Prior to curing, the gasket-forming material is capable of flow, though generally viscous flow, which flow is sufficient to facilitate the screen printing process. The gasket-forming material is also sufficiently adhesive to adhere either to the plate to which it will be applied or to an underlying first layer of gasket material.

In one version of the screen printing process, a first layer of gasket material is printed onto the plate and then cured. After this first printing, a second layer of gasket material is overlaid on the first, a smooth platen of appropriate shape (generally very flat) is overlaid upon the printed gasket material so that a uniform weight is applied to the printed gasket material (while taking precautions to prevent destructive adhesions of gasket material to the platen such as described further below), and the gasket material is cured. The use of two printings of gasket-forming material helps form a foundation of gasket material prior to the smoothing process effected after the second printing and to achieve the needed smoothness and uniform thickness of the sealing surface of the gasket. To achieve this needed smoothness and uniform thickness, it is important to apply a sufficiently uniform pressure to the gasket during a final curing process. This pressure should be selected to be, for the particular gasket-applying process, sufficiently high to create the needed uniformity during the curing process, but not so high as to overly compress cured portions of gasket material such that upon release of the pressure these portions re-expand and create a non-uniform seal thickness. A si gle print process can also be used, and such a single print process is generally preferred since it is simpler and more readily applied to a production process. In a single print process, which is described further below, a platen is applied directly after the first (and only) printing of gasket material, and prevented from settling down too far or too unevenly by mechanical stops.

Preferably, the width of each print feature on the screen is uniform, as width non-uniformities increase the probability of a thickness non-uniformity at the end of the

process. Figure 6B shows an illustrative print screen pattern, wherein gasket material is applied between the closely spaced (here, for example 6 mils) lines. After printing and processing, the applied gasket patterns are broadened. For example, in applications using the two-print process and 6 mil wide pattern on the print screen, an 18 mils wide pattern has been produced. In Figure 6A, the fifty figure eight patterns outlined in dark lines represent the gasket about one hundred reaction wells on a reaction cell plate, with the individual wells (not shown) located within the two openings in the illustrated figure eight patterns. In Figure 6B is illustrated a print screen pattern used to generate one of the figure eight patterns. In another embodiment, each individual reaction well has an O-shaped gasket pattern about it. This latter embodiment avoids the gasket boundary shared by two reaction wells in the pattern of Figures 6A and 6B. This shared boundary can be more susceptible to non-uniformities than the other boundaries of the pattern. The dimensions illustrated in Figure 6B are in inches.

Illustrative two-print protocol: The plate, in this case a 2 X 2 inch glass plate, is cleaned in a Class 10,000 or, preferably, cleaner cleanroom environment. The plate is inspected under a microscope for lint and deposits. These are removed with tweezers and by a stream of propanol or other solvent. The plate is wiped with a lint-free cloth and vapor cleaned with ethanol. After drying, the plate can be stored in a container. The gasket forming material is prepared by degassing the material (e.g., MDX 4-4210™) under vacuum. Care is taken to align the register between the plate and the print screen. The plate can be aligned with three-pin registry with the notches indicated at the edges of the plate illustrated in Figure 6A. The gasket pattern is then printed on the plate, the plate is isolated in a clean container, and the gasket is cured by placing the container in a 70 C oven for 4 hours. Then, the same gasket pattern is overlaid on the first. A thin, preferably transparent, plastic film (for example, a 3 mils thick polyester film) coated with a mold release (for example, 3% wt/v aqueous sodium lauryl sulfate) is layered on top of the printed gasket pattern. Then, a flat, smooth platen is set on top of the release film to evenly apply a weight, for example 2½ lbs., onto the printed gasket pattern. Alternatively, instead of using the film, a mold release agent such as a surfactant can be directly applied to the platen to assure that it does not adhere to the gasket material. The gasket is then cured while the evenly distributed weight is applied. For testing, the release

film is carefully removed, and the gasket examined under a microscope for defects. A clean, smooth plate can be placed on top of the gasket, and a clamping pressure of, for example, 20 lbs. is applied to the gasket pattern of Figure 6A. In a successful print, a contacting interface for each seal segment should be visible. The gasket should be stored in contact with a release film.

Illustrative single-print protocol: The plate is prepared as described above, and a gasket is printed on the plate as described above, taking special care that the gasket-forming material is evenly applied by the print screen. Immediately after this single printing, a transparent, plastic film, which is coated with a release coating, is layered over the printed pattern, and a smooth, flat platen is positioned over the film and the underlying printed pattern. The platen is impressed upon the pattern until it is met by mechanical stops that hold the platen at a uniform height above the plate. The gasket is then cured while the platen is in place. The gasket can be tested and stored as described above.

The screen used in the printing can be formed for example using conventional photolithographic means. Thus, it can be the same type of screen as those used in the manufacture of printed circuit boards. The screen is for example woven from 0.9 mil stainless steel wire. The weave pattern is preferably oriented at about a 45 degree angle from the printing (squeegee) direction. The photolithographic emulsion on which the pattern is made in can be, for example, 2.5 mils thick. With the 6 mil screen pattern width illustrated in Figure 6B and the 2.5 mil screen pattern depth, the gasket width after curing is typically about 18 mil and the thickness of the seal is typically about 1.3 mils. In printings using MDX 4-4210, a typical product gasket has a hardness of about 65 durometer.

The width and thickness of the gasket can be varied by, for example, varying the dimensions of the screen pattern, varying the size of emulsion particles in the polymeric component of the gasket-forming material, varying the weight applied in the curing process, and adding additives to the gasket-forming material such as an inert filler.

It should be recognized that the gasket-forming process, while preferably applied to the flat plates contemplated in the preferred embodiments of the liquid distribution system, can also be applied to any other surfaces to which a complementary surface can be sealed via the gasket. Generally, such other surfaces will be sufficiently smooth so as to facilitate printing of the gasket and sealing to the complementary surface.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations in the preferred devices and methods may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims that follow.

What is claimed:

1. A plate, optionally rectangular, having a first edge and a second edge and having a plurality uniformly sized reaction cells, optionally arrayed in rows and columns, formed in its upper surface, wherein the density of the reaction cells is at least about 10 cells per cm^2, and optionally no more than about 350 per cm^2.

2. The plate of claim 1, wherein the density of cells is from about 10 cells per cm^2 to about 45 cells per cm^2, and each cell has a seal strip width from about 300 μm to about 1,000 μm, wherein optionally the density is from about 10 cells per cm^2 to about 20 cells per cm^2, and each cell has a seal strip width from about 600 μm to about 1,000 μm.

3. The plate of one of claims 1 and 2, wherein the pitch between reaction cells in a row or column is at least about 0.5 mm, optionally wherein each reaction cell is separated from each adjacent reaction cell by at least about 0.15 mm.

4. The plate of claim 3, wherein the diameter or width of an aperture of the cells is about 400 μm to about 1100 μm , and optionally a depth of the cells is from about 100 μm to about 400 μm.

5. The plate of one of claims 1 and 2, further comprising a first marker on the first edge of the plate, wherein the first marker is for orienting the reaction cells, optionally further comprising a second marker on the second edge of the plate perpendicular to the first edge, wherein the second marker is for orienting the reaction cells.

6. The plate of claim 5, wherein the first and second markers are notches designed to interact with locating pins used to mechanically orient the reaction cells, wherein optionally the plate further comprises a third notch for interacting with locating pins.

7. The plate of claim 5, wherein the first and second markers are optical reference structures for orienting a device relative to the reaction cells, wherein optionally the optical reference structures are etched into the plate.

8. The plate of one of claims 1 and 2, further comprising a patterned gasket on its upper surface.

9. A reaction system for conducting a plurality of reactions in parallel, the reaction system comprising:

a liquid distribution system for addressably directing a plurality of liquids to a plurality of cells, and

a plate according to one of claims 1 and 2 comprising said cells.

10. A method of conducting a plurality of reactions in parallel comprising:

operating a liquid distribution system for addressably directing a plurality of liquids to a plurality of cells, wherein the cells are located on a plate according to one of claims 1 and 2 that is reversibly sealed to the liquid distribution system.

11. A method of forming a gasket on the top surface of a substrate, the method comprising:

patterning a layer of photoresist on the top surface so that there are cleared surface areas and photoresist-coated areas;

applying an elastomeric gasket material, optionally silicone, to the cleared areas; and

removing the photoresist from the photoresist-coated areas.

12. The method of claim 11, wherein the applying step comprises:

placing the elastomeric gasket material to the top surface; and

compression-molding the gasket material into the cleared areas, wherein optionally after the compression-molding and before the photoresist removal, the gasket material is cured.

13. A method of forming a gasket comprising:

(a) screening printing a curable polymeric material onto a surface of a substrate so as to form a gasket pattern that can be used to prevent fluid inflow and outflow from an area of the surface;

(b) applying a platen to the top surface of the printed gasket pattern, optionally with the platen impressed upon the printed gasket pattern until it encounters mechanical stops and optionally the platen is applied so as to apply a uniform weight to the printed gasket pattern; and

(c) curing the printed polymeric material to obtain the gasket.

14. The method of claim 13, further comprising, after step (a) but before step (b):

(d) first, curing the polymeric material of the printed gasket pattern; and

(e) second, adding to the printed gasket pattern by overlaying a second screen print of curable polymeric material onto the printed gasket pattern to increase the amount of polymeric material in the printed gasket pattern.

15. The method of one of claims 13 and 14, wherein the uniformly applied weight is between about 0.5 lbs per in^2 and about 6 lbs. per in^2.

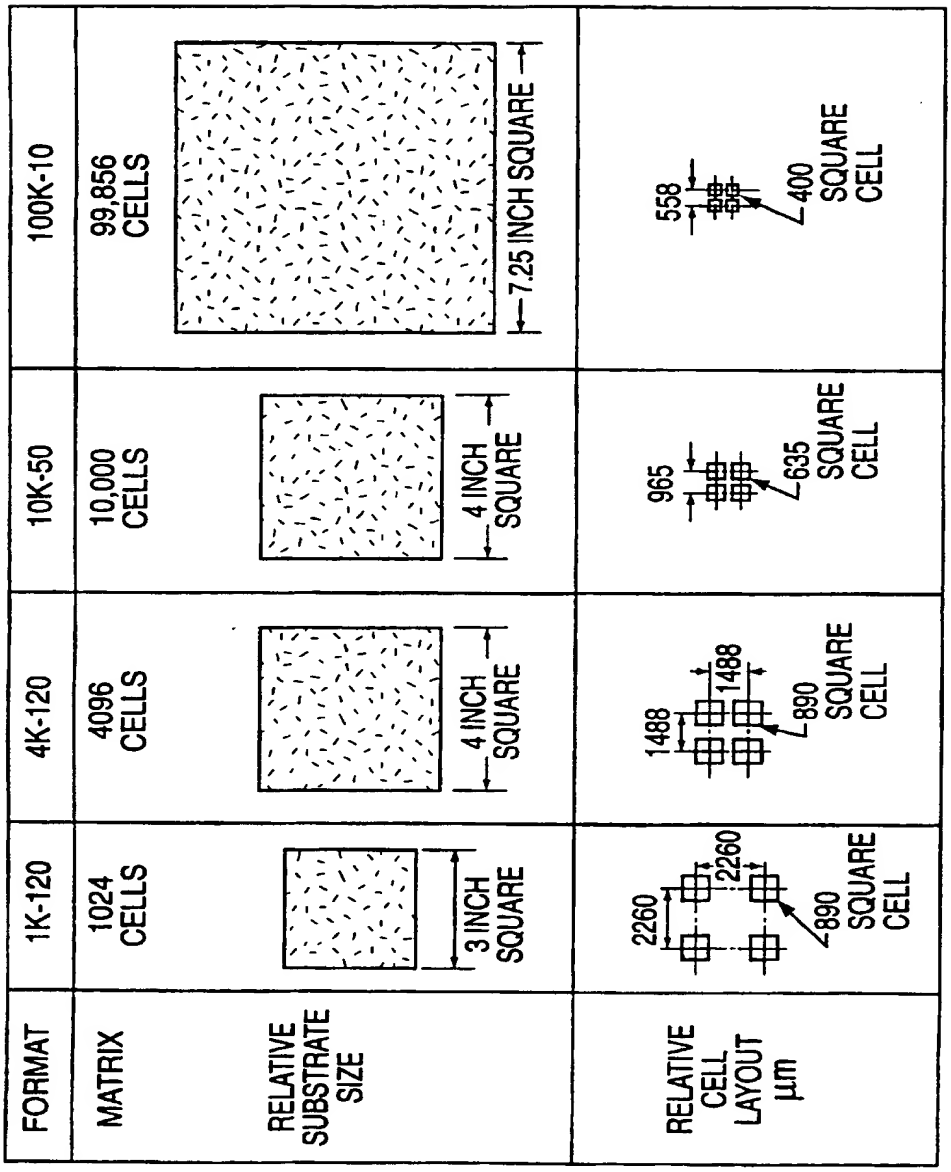
FORMAT
MATRIX
RELATIVE SUBSTRATE SIZE
RELATIVE CELL LAYOUT µm
1K-120
1024 CELLS
3 INCH SQUARE
2260
2260
890 SQUARE CELL
4K-120
4096 CELLS
4 INCH SQUARE
1488
1488
890 SQUARE CELL
10K-50
10,000 CELLS
4 INCH SQUARE
965
635 SQUARE CELL
100K-10
99,856 CELLS
7.25 INCH SQUARE
558
400 SQUARE CELL

FIG. 1

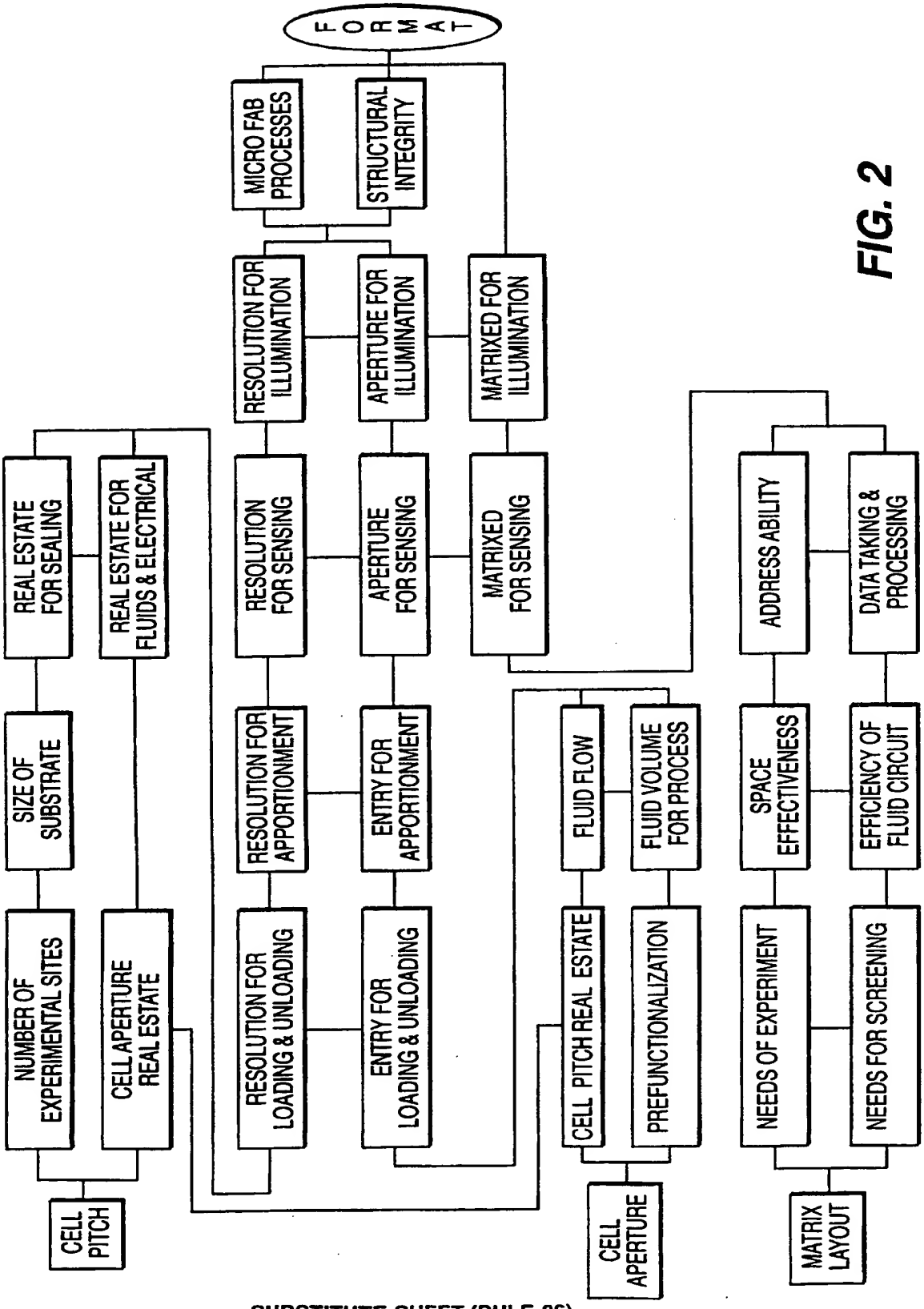
FORMAT
MICRO FAB PROCESSES
STRUCTURAL INTEGRITY
RESOLUTION FOR ILLUMINATION
APERTURE FOR ILLUMINATION
MATRIXED FOR ILLUMINATION
REAL ESTATE FOR SEALING
REAL ESTATE FOR FLUIDS & ELECTRICAL
RESOLUTION FOR SENSING
APERTURE FOR SENSING
MATRIXED FOR SENSING
ADDRESS ABILITY
DATA TAKING & PROCESSING
SIZE OF SUBSTRATE
RESOLUTION FOR APPORTIONMENT
ENTRY FOR APPORTIONMENT
FLUID FLOW
FLUID VOLUME FOR PROCESS
SPACE EFFECTIVENESS
EFFICIENCY OF FLUID CIRCUIT
NUMBER OF EXPERIMENTAL SITES
CELL APERTURE REAL ESTATE
RESOLUTION FOR LOADING & UNLOADING
ENTRY FOR LOADING & UNLOADING
CELL PITCH REAL ESTATE
PREFUNCTIONALIZATION
NEEDS OF EXPERIMENT
NEEDS FOR SCREENING
CELL PITCH
CELL APERTURE
MATRIX LAYOUT

FIG. 2

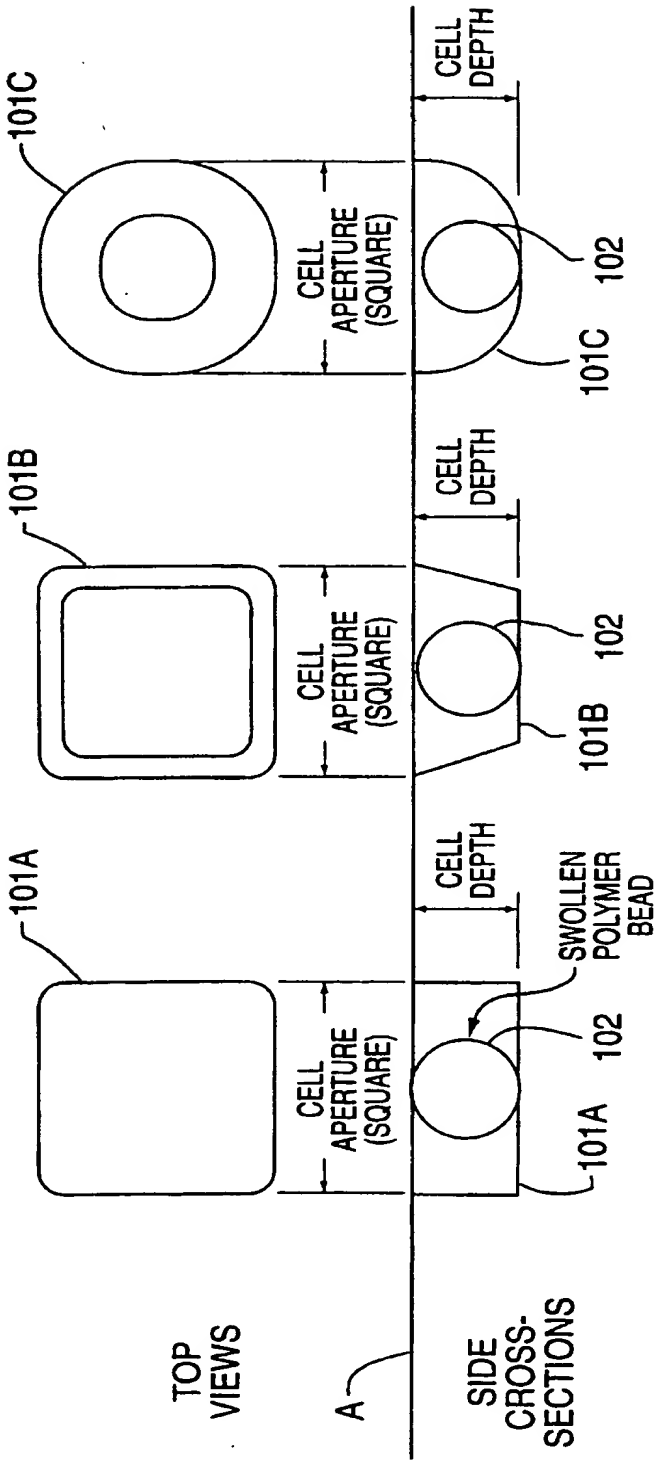
101C
CELL APERTURE (SQUARE)
CELL DEPTH
102
101C
101B
CELL APERTURE (SQUARE)
CELL DEPTH
102
101B
101A
CELL APERTURE (SQUARE)
CELL DEPTH
SWOLLEN POLYMER BEAD
102
101A
TOP VIEWS
A
SIDE CROSS-SECTIONS

FIG. 3

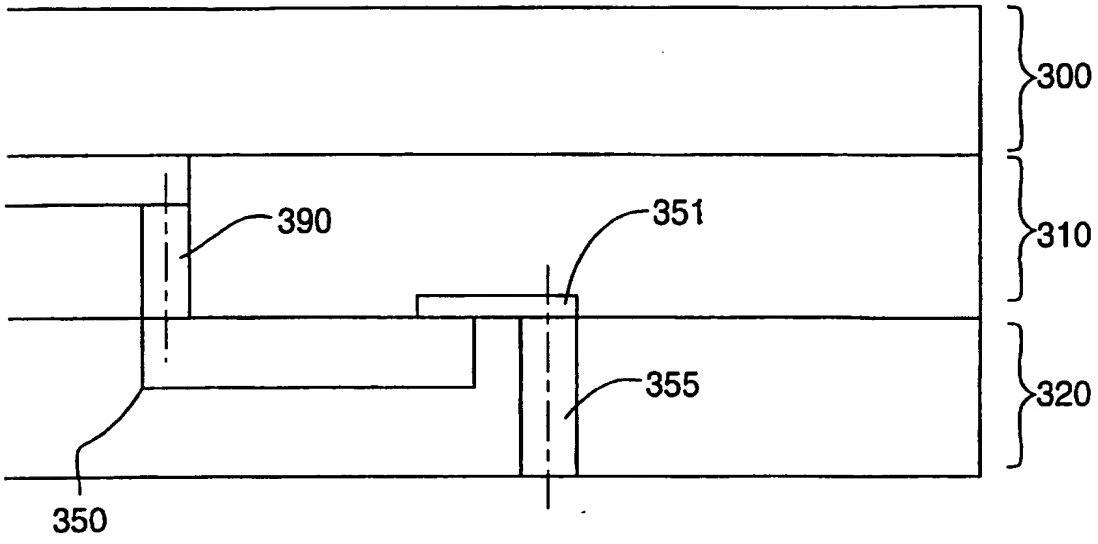
300
310
320
390
351
355
350

FIG. 4

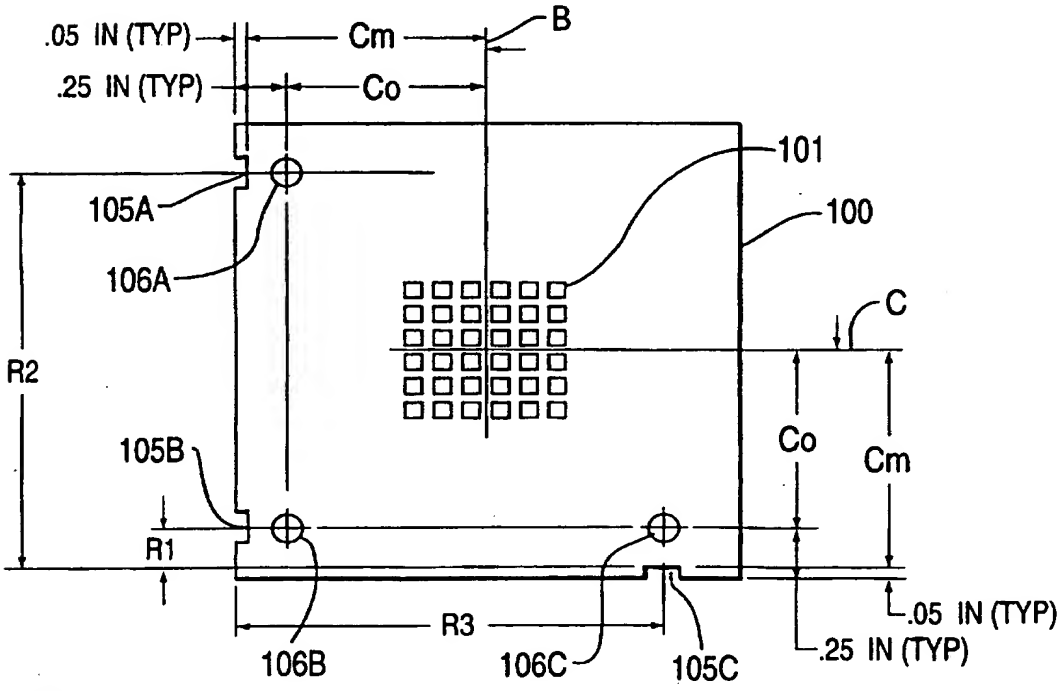
.05 IN (TYP)
.25 IN (TYP)
Cm
Co
B
101
100
105A
106A
C
R2
105B
Co
Cm
R1
R3
106B
106C
105C
.05 IN (TYP)
.25 IN (TYP)

FIG. 5

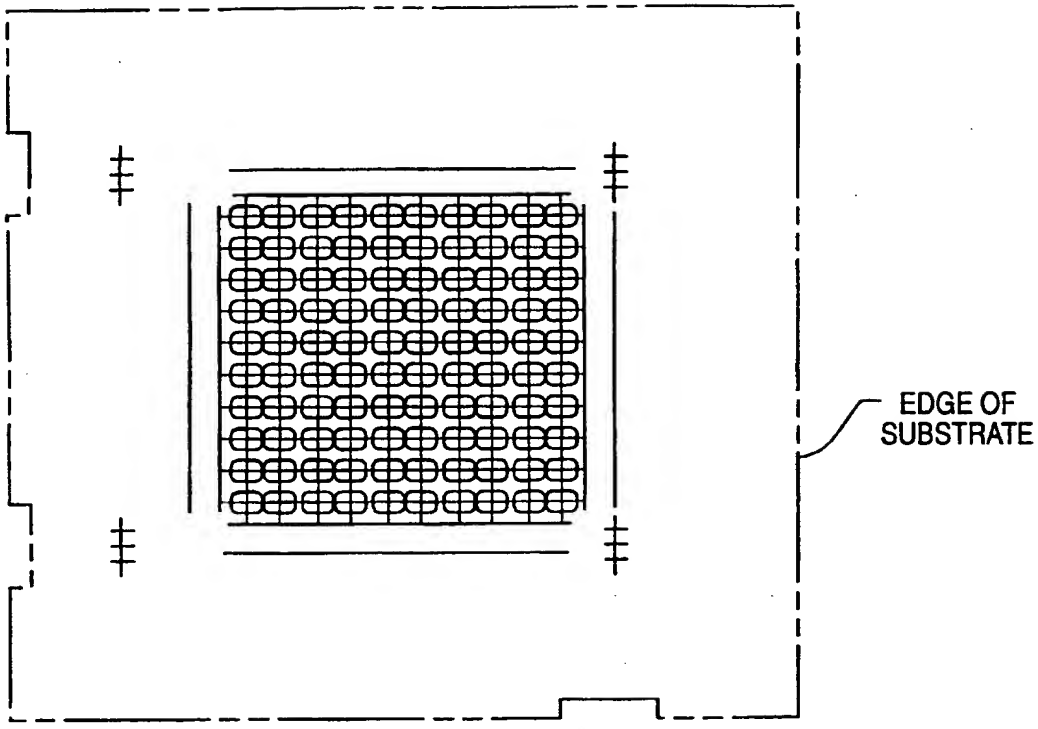

FIG. 6A

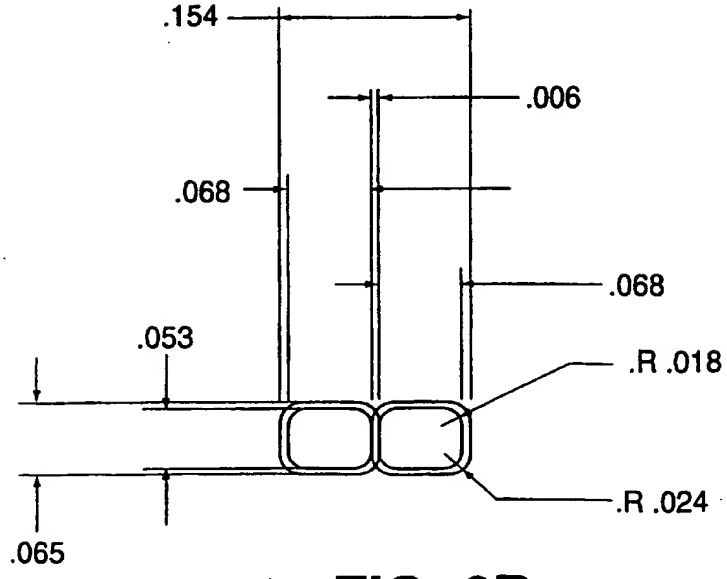

FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05841

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :B01J 19/00
US CL :422/102
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/82.5, 102, 101, 102, 103, 104; 356/246, 427, 440; 204/299R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y P	US 5,529,756 A (BRENNAN) 25 June 1996, see entire document.	1-15
Y	US 4,908,112 A (PACE) 13 March 1990, see entire document.	1-15
A	US 5,453,359 A (GARGAN et al) 26 September 1995, see entire document.	1-15
A	US 5,256,376 A (CALLAN et al) 26 October 1993, see entire document.	1-15
A	US 5,038,852 A (JOHNSON et al) 13 August 1991, see entire document.	1-15
A	US 4,966,646 A (ZDEBLICK) 30 October 1990, see entire document.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 JUNE 1997	Date of mailing of the international search report 05 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HAROLD Y. PYON Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05841

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,891,120 A (SETHI et al) 02 January 1990, see entire document.	1-15